Effect of powder source and processing conditions on the solubility of milk protein concentrates 80

By V. SIKAND[1], P.S. TONG*[1], S. VINK[1] and J. WALKER[2]

[1]Dairy Products Technology Center and
[2]Department of Statistics, California Polytechnic State University San Luis Obispo, CA 93407
*E-mail: ptong@calpoly.edu

Two milk protein concentrates powders with 80% protein content (MPC80) were reconstituted with either water or permeate at 4°C or 37°C to contain 3.5% protein content. Samples were homogenized at 0 or 13800 kPa. The objective of this research was to determine how different solubility measures such as solubility index, soluble solids and particle size were affected by powder source, reconstitution solvent type, temperature of reconstitution and homogenization. A separate analysis of variance was run with each solubility measure as a response. The relationship between treatment effects on the results for solubility index and mean particle size were the most similar. A combination of solvent type, temperature, and homogenization affected both the solubility index and mean particle size significantly. Powder source also affected solubility and particle size and the effect of powder source was not modified by any other factors. These studies indicate that the reconstitution temperature and homogenization can be exploited to improve MPC80 solubility. In this study, reconstituting MPC80 at a temperature of 37°C followed by homogenization at 13800 kPa resulted in highest solubility of MPC80.

Zum Einfluss der Pulverherkunft und der Prozessbedingungen auf die Löslichkeit von Milcheiweiß-Konzentraten 80

Zwei Milcheiweiß-Konzentratpulver mit 80% Eiweiß (MCP 80) wurden entweder mit Wasser oder mit Permeat bei 4 bzw. 37°C auf einen Eiweißgehalt von 3,5% rekonstituiert. Die Proben wurden bei 0 oder 13.800 kPa homogenisiert. Es war die Zielsetzung dieser Studie zu ermitteln, wie unterschiedliche Messgrößen der Löslichkeit wie Löslichkeitsindex, lösliche Feststoffe und Partikelgröße durch die Herkunft des Pulvers, die Art des Lösungsmittels zur Rekonstitution, die Rekonstitutionstemperatur und die Homogenisierung beeinflusst werden. Eine getrennte Varianzanalyse wurde bezüglich jeder Messgröße der Löslichkeit durchgeführt. Die Beziehungen zwischen den Auswirkungen der Behandlungen auf die Ergebnisse des Löslichkeitsindexes und der durchschnittlichen Partikelgröße waren sehr ähnlich. Eine Kombination aus Art des Lösungsmittels, Temperatur und Homogenisa-tionsdruck beeinflusste sowohl den Löslichkeitsindex wie auch die durchschnittliche Partikelgröße signifikant. Die Pulverherkunft beeinflusste die Löslichkeit und die Partikelgröße, die Auswirkung der Pulverherkunft wurde nicht durch andere Faktoren modifiziert. Diese Untersuchung hat gezeigt, dass die Rekonstitutionstemperatur und die Homogenisierung herangezogen werden können, um die Löslichkeit von MPC 80 zu verbessern. In dieser Studie führte die Rekonstitution von MPC 80 bei einer Temperatur von 37°C, gefolgt durch eine Homogenisierung bei 13.800 kPa, zur besten Löslichkeit des Pulvers.

75 Milk protein concentrate (influencing factors of solubility)

75 Milcheiweiß-Konzentrat (Einflussfaktoren der Löslichkeit)

1. Introduction

Milk protein concentrate (MPC) is manufactured from skim milk by ultrafiltration, and diafiltration followed by spray drying (10). The ultrafiltration process results in the concentration of protein and removal of lactose, minerals and water from skim milk. The diafiltration process results in further removal of lactose and minerals. The processing steps used during the manufacture of MPC can result in significant changes in the environment of the milk proteins. These changes in the milk protein environment may adversely affect the physicochemical state of the protein molecules and hence its functional properties (11). MCKENNA (9) reported that during MPC processing, specifically at the diafiltration stage, loss of k-casein from the micelles reduced electrostatic repulsion and increased casein aggregation.

Previous studies (3, 8 and 9) have attempted to characterize the protein interactions in the insoluble material in MPC. MCKENNA (9) reported that insoluble material in MPC85 consisted of fused casein micelles held together by protein-protein interactions. HAVEA (8) confirmed the MCKENNA (9) findings and reported that insoluble material mainly consisted of alpha-caseins and beta-caseins that interact via hydrophobic protein-protein interactions. ANEMA (3) reported that the development of higher insolubility over storage time involved the formation of lactosylated casein as a result of Maillard reactions. It was postulated that enhanced cross-linking of surface proteins was the probable reason of insoluble material in high protein MPC.

In a survey on basic functionality of 37 different MPC products from 10 different countries, poor solubility for high milk protein powder (protein content ranging from 82% to 86%) was reported (6). Degree of protein solubility of food ingredients may depend on environmental factors such as temperature, ionic strength of the solvent, time of contact, pH and mechanical shear (14). Recently, GUALCO (12) found that solubility of MPC80 can be increased by adding NaCl to diafiltration water during MPC processing. Thus, minerals can play an important role in the solubility of MPC80.

Attaining quicker and more complete solubility of MPC80 would enhance the functional properties and

hence, the use of high protein powders. The aim of this research was to determine the impact of the type of solvent (permeate or water) used for reconstitution, reconstitution temperature (4°C or 37°C) and homogenization pressure (0 kPa or 13800 kPa) on the solubility of commercially available MPC80 powders. Because there is no standard protein solubility test, the present study uses three measures of solubility to correlate the results from two different commercial sources of MPC80 powders. The solubility tests are: Solubility index (SI), a traditional measure of protein insolubility; percent soluble solids (%S), an important measure indicative of stability of protein dispersion or absence of sedimentable material; and mean particle size (MPS), an important measure indicative of aggregation or dissociation of particles.

2. Materials and methods

2.1 Materials

MPC powders: Commercial samples of readily available MPC80 were obtained from two sources, and are identified as MPC80-M1 and MPC80-M2.

Permeate: Pasteurized skim milk was ultrafiltered to produce permeate, which was divided into equal parts. One part was stored at 4°C overnight and was used in the reconstitution of MPC80-M1 and MPC80-M2 samples the following day. The other part was immediately used to reconstitute MPC80 at 37°C. In our experiments, approximately 0.262 Kg and 0.280 Kg of MPC80 were reconstituted per 6 Kg of water or permeate to achieve 3.5% protein each for MPC80-M1 and MPC80-M2 solutions. Reconstitution was performed by adding powder to the solvent under high shear mixing using a Silverson high shear mixer (Model DX60) operating at 30 Hz for 20 min. After reconstitution, half of each reconstituted MPC80 solution was homogenized at a pressure of 13800 kPa; the other half was passed through a homogenizer at 0 kPa. The temperature of homogenization was 37°C.

Experimental design: The experimental design is detailed in Fig. 1. The experiment was replicated three times.

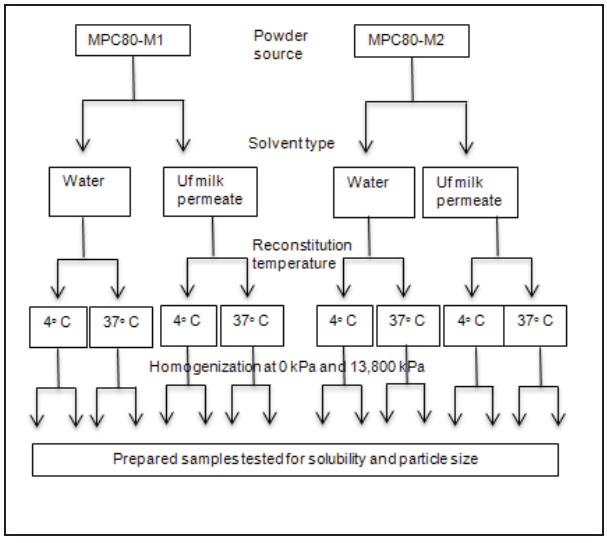

Fig 1: Schematic of treatments applied to MPC80 powders to test solubility

2.2 Methods

Composition analysis: Compositional analysis was done by standard AOAC methods (2).

Solubility methods (*SI)*: The original SI method developed for NFDM solubility (1) was modified for MPC80 by increasing the centrifugation time from 5 to 10 min.

%S: Aliquots of all the prepared samples were centrifuged at 700 x *g* for 10 min at 20℃. The total solids (TS) of supernatants and original prepared samples were determined by keeping aliquots of samples in oven at 100°C for 20 h and upon cooling, the TS content was measured (3). The %S was calculated by measuring TS in supernatants divided by original solution TS, multiplied by 100.

MPS: The particle size distribution of MPC80 reconstituted samples was measured by using LS230 laser diffraction particle size analyzer (Beckman Coulter, Miami, FL). The analyzer was in the polarization intensity differential scattering optical mode, which enhances the detection resolution of particles ≤0.8 μm. The MPS (μm) was determined by taking average diameters d50, which means 50% of the particles had a larger diameter and other 50% had lower diameter. Samples reconstituted with water and permeate at 4°C or 37°C and homogenized at 0 kPa and 13800 kPa were analyzed the same day.

Statistical methodology: The data were analyzed with analysis of variance (ANOVA) using PROC MIXED in SAS version 9.1. Each solubility measure was the response in a separate ANOVA. The factors in each ANOVA were identical—powder source, solvent type, reconstitution temperature, and homogenization. The ANOVA model included all the main effects and their interactions as well as random effects for replicate and sample differences. Because each ANOVA contained a large number of main effects and interactions, each term was evaluated using a 1% significance level. If significant differences were found, all pairs of levels from the highest-order significant main effect or interaction were compared using Tukey's HSD method—also with a 1% significance level.

3. Results and Discussion

3.1 MPC80 Composition

Compositional analyses of the two commercially-obtained MPC80 powders are shown in Table 1.

Table 1: Compositional analyses of two commercial MPC80 powders

Component	Powder source (Mean±SD, %)	
	MPC80-M1	MPC80-M2
Protein	75.0±1.21	80.00±1.00
Moisture	4.29±0.20	4.42±0.34
Fat	1.59±0.08	1.35±0.30
Ash	6.21±0.02	7.42±0.06

The average total protein content (total nitrogen x 6.38) observed for MPC80-M1 and MPC80-M2 was 75% and 80%, respectively. Ash values observed for both MPC80-M1 and MPC80-M2 samples were 6.21% and 7.42%, respectively. The higher ash content of MPC80-M2 may be attributed partly to higher protein as casein protein is associated with more cal-

cium and phosphorus. Furthermore, data in our lab shows that adding salt during the diafiltration stage of MPC manufacturing (12) resulted in higher ash content. Moisture content for both MPC80-M1 and MPC80-M2 samples was observed to be 4.29% and 4.42% respectively. The fat content for both MPC80-M1 and MPC80-M2 samples was observed to be 1.59 and 1.35%, respectively.

3.2 Solubility

SI: A higher sedimentation volume in the centrifuge tube represents lower solubility and vice versa. High SI for MPC80 has also been reported (11, 12).

Table 2: P-values for ANOVAs of different solubility measures (solubility index (SI), % soluble solids (%S) and mean particle size (MPS))

Fixed effect	Response variable		
	SI	%S	MPS
PS	<.0001	<.0001	<.0001
ST	<.0001	<.0001	<.0001
PS * ST	0.0962	<.0001	0.0880
RT	<.0001	<.0001	<.0001
PS * RT	0.6836	0.0002	0.0130
ST * RT	0.4525	0.0012	0.0005
PS * ST * RT	0.7707	0.0035	0.2444
H	<.0001	<.0001	<.0001
PS * H	0.4509	<.0001	0.1445
ST * H	<.0001	<.0001	0.2359
PS * ST * H	0.0539	<.0001	0.1668
RT * H	<.0001	<.0001	0.1224
PS * RT * H	0.0753	0.2830	0.9535
ST * RT * H	<.0001	<.0001	<.0001
PS * ST * RT * H	0.2518	<.0001	0.2114

PS-Powder source, ST-Solvent type, RT-Reconstitution temperature, H-Homogenization

Table 3: Mean solubility index (SI) for reconstituted MPC80-M1 and MPC80-M2

Powder source	Mean SI (ml)
MPC80-M1	4.61[a]
MPC80-M2	2.95[b]

Samples sharing the same letter have no statistically significant differences; α = 0.01, n = 3. Standard error for individual estimates = 0.0900. Standard error for differences between groups = 0.0701

The ANOVA results in Table 2 show that all four experimental factors affect SI. The effect of powder source on SI is not modified by other factors; however, solvent type, homogenization, and reconstitution temperature all interact to affect SI. Table 3 shows the effect of powder source on SI. MPC80-M1 was observed to have a higher mean SI than MPC80-M2 (p<0.0001). Low SI of MPC80-P2 over MPC80-P1 may be attributed to mineral modification during MPC 80 processing as higher ash content was observed in MPC80-M2 compared with MPC80-M1. These observations are in agreement with studies in our lab that show that by adding NaCl or KCl to the diafiltration water during MPC manufacture results in higher ash content of MPC (treated with salts) compared to control MPC (unpublished data). Solvent type, homogenization, and reconstitution temperature were shown to interact in affecting SI (p<0.0001). Table 4 shows the effect of solvent type, homogenization, and reconstitution temperature on SI. Reconstitution at 37°C with homogenization resulted in the lowest mean SI

Table 4: Mean solubility index (SI) for levels of solvent type, homogenization, and temperature

Solvent type	Homogenization	Temperature (°C)	Mean SI (mL)
Permeate	NO	4	8.25[a]
Water	NO	4	5.75[b]
Permeate	NO	37	4.92[c]
Permeate	YES	4	4.17[d]
Water	YES	4	3.04[e]
Water	NO	37	1.79[f]
Permeate	YES	37	1.30[fg]
Water	YES	37	1.02[g]

Samples sharing the same letter have no statistically significant differences; α = 0.01, n = 3. Standard error for individual estimates = 0.1244. Standard error for differences between groups = 0.1402

levels, which were indistinguishable with 99% overall confidence. Similar effects of increased solubility of micellar casein by increased agitation and temperature were reported (13). Reconstitution of MPC80-M1 and MPC80-M2 with permeate at 4°C without homogenization resulted in the highest mean SI level and with water at 4°C without homogenization resulted in the second highest mean SI level.

%S: The ANOVA in Table 2 shows that powder source, solvent type, homogenization, and temperature were shown to interact in affecting the %S (p < 0.0001). Table 5 shows the effect of these factors on %S. Similar to our results for SI, MPC80-M2 reconstituted with either permeate or water at 37°C followed by homogenization, had among the highest %S. MPC80-M1 reconstituted with permeate at 37°C with homogenization was also among the most soluble, but when reconstituted with water, MPC80-M1 had significantly lower mean %S. Also, temperature seemed to have a lesser effect on solubility in MPC80-M2. MPC80-M2 reconstituted with permeate or water at 4°C and homogenized also had among the highest levels of mean %S.

The lowest mean %S occurred when MPC80-M1 was reconstituted with water at 4°C without homoge-

Table 5: Mean percent soluble solids (%S) for levels of powder source, solvent type, homogenization, and temperature

Powder source	Solvent type	Homogenization	Temperature (°C)	%S
MPC80-M2	Permeate	YES	37	100.00[a]
MPC80-M2	Water	YES	37	99.83[ab]
MPC80-M2	Permeate	YES	4	97.60[abc]
MPC80-M1	Permeate	YES	37	97.52[abc]
MPC80-M2	Water	YES	4	97.30[abc]
MPC80-M2	Permeate	NO	37	94.96[bc]
MPC80-M1	Water	YES	37	93.43[cd]
MPC80-M2	Water	NO	37	92.41[cd]
MPC80-M1	Permeate	YES	4	88.95[de]
MPC80-M1	Water	YES	4	88.75[de]
MPC80-M2	Permeate	NO	4	84.67[ef]
MPC80-M2	Water	NO	4	81.17[fg]
MPC80-M1	Permeate	NO	37	80.29[fg]
MPC80-M1	Water	NO	37	77.57[gh]
MPC80-M1	Permeate	NO	4	73.33[h]
MPC80-M1	Water	NO	4	51.52[i]

Samples sharing the same letter have no statistically significant differences; α = 0.01, n = 3. Standard error for individual estimates = 0.8723%. Standard error for differences between groups = 1.2305%. The five samples that were statistically indistinguishable for producing the highest mean percent soluble solids were all homogenized (they share letter *a*). None of the 6 least soluble samples were homogenized (they share letters *f, g, h* or *i*) n=3

nization. MPC80-M1 reconstituted with permeate at 4°C without homogenization was significantly more soluble, but still among the least soluble samples. It has been reported that MPC80 samples treated with salt were more soluble at room temperature (5, 12) even after storage of powders at 40°C as compared to solubility of untreated MPC samples (5). It may be possible that MPC80-P2 was processed differently (with salt) to enhance solubility.

Fresh MPC85 powder samples were reported (8) to have solubility of 53% at low temperature (20°C). Our results indicate that reconstitution of MPC80 at 37°C combined with homogenization increases the MPC80 dispersion. In addition, permeate contains mineral salts, especially more potassium and sodium salt besides lactose and non-protein nitrogen. It has been reported that salts (NaCl and KCl) enhance the solubility of MPC80 (5, 12) probably by decreasing hydrophobic interaction.

MPS: The ANOVA results in Table 2 show that all four experimental factors affect MPS. The effect of powder source on MPS is not modified by other factors; however, solvent type, homogenization, and temperature all interact to affect MPS. Table 6 shows the effect of powder source on MPS. MPC80-M1 was observed to have a higher average MPS than MPC80-M2 ($p < 0.0001$).

Table 6: Average of mean particle size (MPS) for levels of powder source

Powder source	Mean particle size (µm)
MPC80-M1	72.19 [a]
MPC80-M2	40.18 [b]

Samples sharing the same letter have no statistically significant differences; α = 0.01, n = 3. Standard error for individual estimates = 1.7498. Standard error for differences between groups = 2.4746

Table 7: Average of mean particle size (MPS) for levels of solvent type, homogenization, and temperature

Solvent type	Homogenization	Temperature (℃)	Mean MPS (µm)
Water	NO	4	96.06[a]
Permeate	NO	4	80.95[ab]
Water	YES	4	72.65[bc]
Water	NO	37	72.12[bc]
Permeate	NO	37	49.94[c]
Permeate	YES	37	28.51[d]
Water	YES	37	27.16[d]
Permeate	YES	4	22.11[d]

Samples sharing the same letter do not have statistically significant differences; α = 0.01, n = 3. Standard error for individual estimates = 3.2413. Standard error for differences between groups = 4.4891

Solvent type, homogenization, and temperature were shown to interact in affecting MPS ($p < 0.0001$). Table 6 shows the effect of solvent type, homogenization, and temperature on the MPS. Similar to the results for SI, samples reconstituted with either permeate or water at 37°C and homogenized were statistically among the lowest (Table 7) in their average MPS. Samples reconstituted with either permeate or water at 4°C without homogenization were among the highest (Table 7) in their average MPS. However, those samples reconstituted at 4°C with homogenization showed very different MPS depending on whether they were reconstituted with permeate or water. Samples reconstituted with permeate at 4°C and homogenized were among the lowest in average MPS, but samples reconstituted with water at 4°C and homogenized were among the second highest in average MPS (Table 7). The difference in the average MPS for these groups was estimated to be almost 44 µm.

Our results for non-homogenized conditions for MPC80-M1 and MPC80-M2 were found to be in agreement with the results of BELICIU et al. (4). These authors reported a decrease in size of casein micelles using ultrafiltered permeate as a solvent and increasing temperature to 50°C. FERRER et al. (7) reported that particles greater than 30 mm in diameter "will eventually sediment under quiescent conditions" and that heating at 40°C and 60°C for 30 minutes and shear was essential to completely disperse the particles. Our studies show that the reconstitution temperature of 37°C and homogenization can be used to improve MPC80 solubility.

4. Conclusions

The solubility of MPC80 is affected by solvent type, temperature of reconstitution, and presence or absence of homogenization, while homogenization improved solubility. Homogenization produced a product with smaller MPS, thus facilitating the reconstitution. In almost all cases, samples of MPC80 reconstituted at 37°C were more soluble than the same samples reconstituted at 4°C. Our results show that MPC80 obtained from different sources exhibit differences in solubility. However, we observed a similar trend of enhanced solubility in both powders by increasing temperature, homogenization and solvent type.

5. References

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